

On-probe pyrolysis desorption electrospray ionization (DESI) mass spectrometry for the analysis of non-volatile pyrolysis products

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Abstract

An on-probe pyrolyzer has been constructed and interfaced with desorption electrospray ionization (DESI) mass spectrometry (MS) for the rapid analysis of non-volatile pyrolysis products. The detection and analysis of non-volatile pyrolysis products of peptides, proteins and the synthetic polymer poly(ethylene glycol) were demonstrated with this instrument. The on-probe pyrolyzer can be operated off-line or on-line with the DESI source and was interfaced with a tandem MS (MS/MS) instrument, which allowed for structure characterization of the non-volatile pyrolytic products. Advantages of this system are its simplicity and speed of analysis since the pyrolysis is performed *in situ* on the DESI source probe and hence, it avoids extraction steps and/or the use of matrices (e.g., as in MALDI-MS analyses).

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1. Introduction

Pyrolysis mass spectrometry (Py-MS) has been extensively used to identify the pyrolytic products of synthetic polymers, with gas chromatography–(electron ionization)-MS historically being the main technique for the analysis of volatile pyrolytic products [1]. The development of soft ionization sources like electrospray ionization (ESI) [2,3] and matrix-assisted laser desorption ionization (MALDI) [4,5] enabled the analysis of high molecular weight polar compounds including proteins and synthetic polymers [6].

Besides offering the ability to analyze the intact synthetic polymer molecules, ESI and MALDI allow the analysis of the non-volatile pyrolysis products of these compounds. MALDI-MS is particularly well suited for the analysis of high molecular weight mixtures and complex synthetic polymer compounds due to the predominant singly charged nature of the signals generated [7]. The use of MALDI-MS to study non-volatile pyrolysis products was first demonstrated with the analysis of pyrolytic products of segmented polyurethane [8]. This study

identified several series of oligomeric non-volatile products over the mass range ~800–10,000 Da, including linear and cyclic polyester oligomers. MALDI-MS was also employed to study low-temperature pyrolysis products from poly(ethylene glycol) [9]. This last study found that the dominant oligomeric products had hydroxyl and ethyl ether end groups, while at higher temperatures, methyl ether and vinyl ether end groups became more abundant in the pyrolyzates. Other studies have also used MALDI-MS for the study of thermal oxidative degradation of nylon-6 [10] and the thermal degradation of aromatic poly(carbonate) polymers in the temperature range of 300–700 °C [11]. Pyrolysis was also combined with MALDI-MS to study the non-volatile pyrolysis products of poly-amino acids and a small protein pyrolyzed in a nitrogen atmosphere and at temperatures ranging from 245 to 285 °C [12]. In this last study, the pyrolysis products were extracted and analyzed by MALDI-MS and it was hypothesized that the amino acid chains undergo dehydration through the formation of cyclic oligopeptides. In addition, the use of ESI-MS for the analysis of non-volatile pyrolysis products was demonstrated with the pyrolysis of dimethylamphetamine [13] and the analysis of thermal decomposition of three common pharmaceuticals: acetaminophen, indomethacin, and mefenamic acid [14]. In all these studies, however, sample preparation was required and involved

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dissolving and extracting the non-volatile residues with appropriate solvents (ESI) or mixing with matrices (MALDI). This sample pre-processing step increases analysis time and could possibly affect the analysis by introducing a sampling bias and consequently not detecting important products.

The introduction of ambient MS techniques has brought a new dimension in mass spectrometric measurements as they allow the analysis of samples in their native environment. To date, a number of ambient ionization methods for MS analysis have been introduced, but most notably are direct analysis in real-time (DART) [15] and desorption electrospray ionization (DESI) [16]. Of interest to this investigation is the ability of DESI to ionize compounds from surfaces with a mechanism similar to conventional ESI and its applicability to analyses of a wide range of molecular weights. These analytes include, but are not limited to, pharmaceuticals and controlled substances [17–19], peptides and proteins [20,21], explosives [22,23], clinical samples [24], intact tissues [25,26], synthetic polymers [27] and bacteria [28]. DESI is a rapid desorption/ionization source for MS and requires little to no sample preparation. DESI is carried out by directing aerosolized and electrosprayed charged droplets and ions of solvent onto the surface to be analyzed. The charged droplets impact on the surface and “pick up” available soluble molecules. These charged droplets subsequently “bounce” at a lower angle towards the MS inlet and yield gaseous ions of the compound in an analogous mechanism to that in ESI [29]. Hence, DESI yields mass spectra similar to those obtained by ESI which are characterized by multiply charged ions and are amenable for tandem mass analysis (MS/MS). However, it is reasonable to assume that the nature and polarity of the DESI solvent can be varied to affect sampling of pyrolysis products during the surface pick up step of the DESI process.

In this article, we describe the development of an on-probe pyrolyzer interfaced to a DESI source as a novel *in situ* and rapid pyrolysis technique to investigate non-volatile pyrolytic residues by MS and MS/MS analyses. The utility of the technique is demonstrated with the analysis of several biological samples and a synthetic polymer.

2. Experimental

2.1. Pyrolysis device and procedures

A diagram of the on-probe pyrolyzer interfaced to the DESI source is shown in Fig. 1. A homebuilt DESI source [21] was interfaced with a quadrupole ion trap MS (LCQ Classic, Thermo Electron, San Jose, CA) and was operated in the positive ion mode. The on-probe pyrolyzer consisted of a membrane heater (Model #HM6815, Minco, Minneapolis, MN) placed underneath a removable glass slide held tightly together with a clamp (Fig. 1b). The sample to be pyrolyzed was placed directly on the center of the glass slide. The membrane heater was powered by alternating current (AC) from a transformer (Model #3PN116C, Superior Electric, Farmington, CT) and heating and final pyrolysis temperature were controlled by adjusting the voltage of the transformer and

the heating time. For our current setup, a voltage of 20 V applied for 11 s resulted in a final pyrolysis temperature of 220 °C. These values for pyrolysis temperature and time were used for all biological samples analyzed in this study. The glass slide surface temperature was measured *in situ* using a thermocouple probe (Model #HH12A, Omega Company, Stamford, CT) placed in direct contact. After sample pyrolysis, the probe was cooled to room temperature (<5 min) and the DESI-MS analysis carried out. This setup is amenable to conducting pyrolysis in either the off-line or on-line mode with the DESI source, that is, a sample placed on a slide can be pyrolyzed in a furnace under controlled atmospheric conditions and later analyzed by DESI-MS. However, all measurements in this report were performed in the on-line configuration (Fig. 1a).

Several model samples were tested with this new on-probe pyrolyzer DESI-MS instrument. Peptides analyzed included Angiotensin II-human, of sequence DRVYIHPF, and the peptide VIP (1–12), of sequence HSDAVFTDNYTR (both from AnaSpec, San Jose, CA). The proteins used were lysozyme and RNase A, and the synthetic polymer used was poly(ethylene glycol) (PEG 2000) (all from Sigma–Aldrich, St. Louis, MO). Methanol, water (from Burdick & Jackson, Muskegon) and tetrahydrofuran (THF, from EMD Chemicals, San Diego, CA) were used for sample preparation and MS measurements (all HPLC grade). About a 1-mg sample of the peptides was dissolved in 200 μ L of methanol, and the entire solution air-dried on a glass slide (covering a surface area approx. 6 cm², \sim 0.1 mg sample/cm²) and placed on the on-probe pyrolyzer. Lysozyme and RNase A were prepared in a similar fashion, but dissolved in water. For poly(ethylene glycol), about 10 mg of PEG 2000 was dissolved in 1 mL of THF, air-dried on a glass slide (\sim 1 mg/cm²), placed on the on-probe pyrolyzer and heated to a final temperature of 250 °C for 30 min.

2.2. DESI and mass spectrometry parameters

The DESI source was operated with a high voltage of 6 kV applied to the spraying solvent. The spraying solvent consisting of 50% methanol in water (v/v) was delivered at a flow rate of 7 μ L/min via a syringe pump. All mass spectra were collected in spectral average mode. The pressure of the DESI nebulizer gas (N₂) was set as 250 psi.

Tandem MS (MS/MS) measurements were conducted with the following parameters: activation q of 0.250; isolation width was 1 amu and the percentage relative collision energy was in the range of 25–40%, and was adjusted to get a precursor ion peak of 25% relative intensity or less (when possible).

3. Results and discussion

The utility and versatility of the DESI source interfaced with the on-probe pyrolyzer for the analysis of non-volatile pyrolysis products were demonstrated with several model compounds that included peptides, proteins and a synthetic polymer.

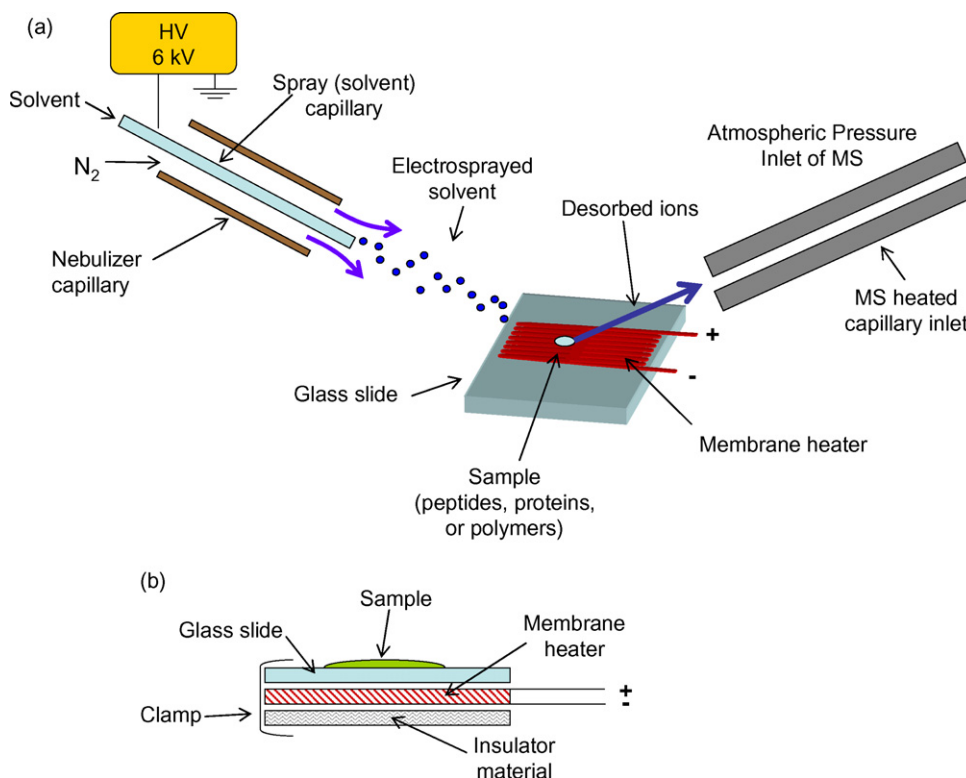


Fig. 1. (a) On-probe pyrolyzer interfaced to the DESI source, (b) detailed diagram of the on-probe pyrolyzer.

3.1. On-probe pyrolysis DESI-MS analysis of biomolecules

Our laboratory previously reported the site-specific pyrolysis-induced cleavage at the amino acid aspartic acid (letter symbol “D”) in both peptides and proteins [30] by heating samples to a temperature of 220–250 °C for 10 s under atmospheric pressure conditions. Peptides and proteins in this previous study were pyrolyzed in an open-ended tube furnace, extracted with a suitable solvent and analyzed by ESI-MS and MS/MS to characterize and identify non-volatile pyrolysis cleavage products. In this report, the same samples were pyrolyzed on-probe and products were analyzed *in situ* by DESI-MS, bypassing the sample extraction, transfer, and ESI-infusion steps. In the ESI-MS study [30] and the DESI-MS study here described, pyrolysis of peptides and proteins above 300 °C produced complete charring of the polypeptide backbone. Our laboratory has detected the pyrolysis induced site-specific cleavage at aspartic acid mostly at low temperature pyrolysis. However, this pyrolysis cleavage reaction is not exclusive in biomolecules as other pyrolysis fragments have been detected and we are currently using the system here described to further characterize the structure and nature of these pyrolysis fragments.

Fig. 2 illustrates the DESI-mass spectra before and after on-probe pyrolysis of the peptide Angiotensin II, along with the tandem mass spectrum of the pyrolytic product at m/z 931. The DESI-mass spectrum of the non-volatile products also shows the formation of a dehydration product at m/z 1028.2, a possible oxidation product at m/z 1124.1 (of yet unknown structure) and the product of the pyrolysis induced site-specific cleavage at

aspartic acid at m/z 931.2 (the D-cleavage pyrolysis peptide product). Tandem MS data of the ion at m/z 931 confirms that sequence-specific information is preserved after low temperature pyrolysis of peptides.

The above measurement demonstrates the simplicity and speed of analysis of pyrolysis residues with the on-probe pyrolyzer coupled to a DESI-MS system. No solvents were required for residue extraction and solubilization, assuring the analysis of the entire pyrolysis product mixture (i.e., the non-volatile fraction, *vide infra*). However, it is reassuring to note that all products detected in the on-probe pyrolysis DESI-MS analysis in Fig. 2 were also observed in the open-ended tube furnace pyrolysis and ESI-MS analysis, which required sample extraction and solubilization [30]. It is important to note that lower MW products like diketopiperazines (DKP) known to be generated under Curie-point [31] and atmospheric [32] pyrolytic conditions were only observed in the analysis of the Angiotensin II peptide (signal at m/z 263 corresponding to the $(M+H)^+$ DKP of VY). This may be due to several factors: first, volatile DKP products may have been lost during the pyrolysis process since the on-probe pyrolyzer is operated at atmospheric pressure. Second, early work on the formation of DKP from dipeptides [33,34] found that only a small percentage (~7%) of the original dipeptide was converted to DKP at 215 °C. And finally, ionization suppression of the DKP $(M+H)^+$ signals within the desorbed DESI droplets may take place, especially if analyzing a complex mixture of pyrolytic products with dissimilar droplet surface activities [35] or DKPs in mixtures with peptides containing highly basic groups (i.e., arginine), as it is the case here.

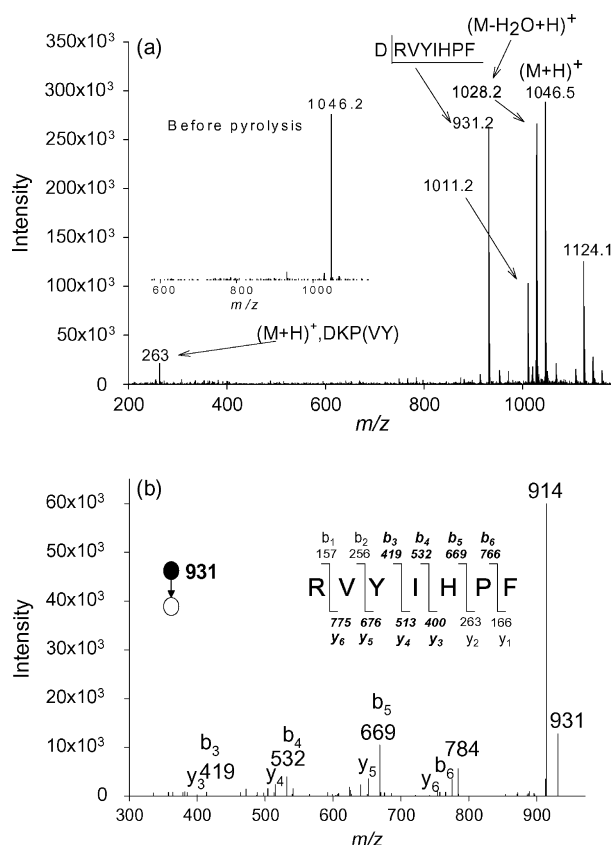


Fig. 2. (a) On-probe pyrolysis (220 °C, 11 s) DESI-mass spectrum of Angiotensin II (inset: before pyrolysis DESI-mass spectrum). Site-specific cleavage is induced at the C-terminus of aspartic acid. Ions at m/z 1028 and 1011 are the result of dehydration and deamination reactions. (b) On-probe pyrolysis DESI-tandem mass spectrum of the ion at m/z 931.

Fig. 3 illustrates the on-probe pyrolysis and DESI-MS analysis of another peptide, VIP (1–12) peptide, which contains two aspartic acid residues. Specifically, the on-probe pyrolysis DESI-mass spectrum (Fig. 3a) is characterized by the ions at m/z 553.6 and 1086.3, which correspond to the expected products due to site-specific cleavages at the two aspartic acid residues (D-cleavage pyrolysis). This D-cleavage pyrolysis is believed to proceed *via* a similar mechanism as in the solution phase reaction, that is, the formation of a five-member cyclic anhydride followed by hydrolysis [36]. Similar results were also obtained in the open-ended tube furnace (at atmospheric pressure conditions) and solvent extraction ESI-MS analysis of the pyrolysis residues [30]. Other ions observed at m/z 's 1068 and 1050 result from consecutive losses of water and ammonia (from arginine) from the pyrolysis fragment at m/z 1086.3, and these ions were also observed in the off-line pyrolysis and extraction ESI-MS measurements. Fig. 3b and c show the on-probe DESI-tandem mass spectra of the pyrolysis products at m/z 553 and 1086, confirming their sequences and the site-specificity of the pyrolysis cleavage at aspartic acid.

Also, the on-probe pyrolysis DESI-MS instrument was used to analyze the non-volatile pyrolysis products of the proteins lysozyme (MW 14.3 kDa) and RNase A (MW 13.7 kDa). Fig. 4 shows the DESI-mass spectra of lysozyme before and after

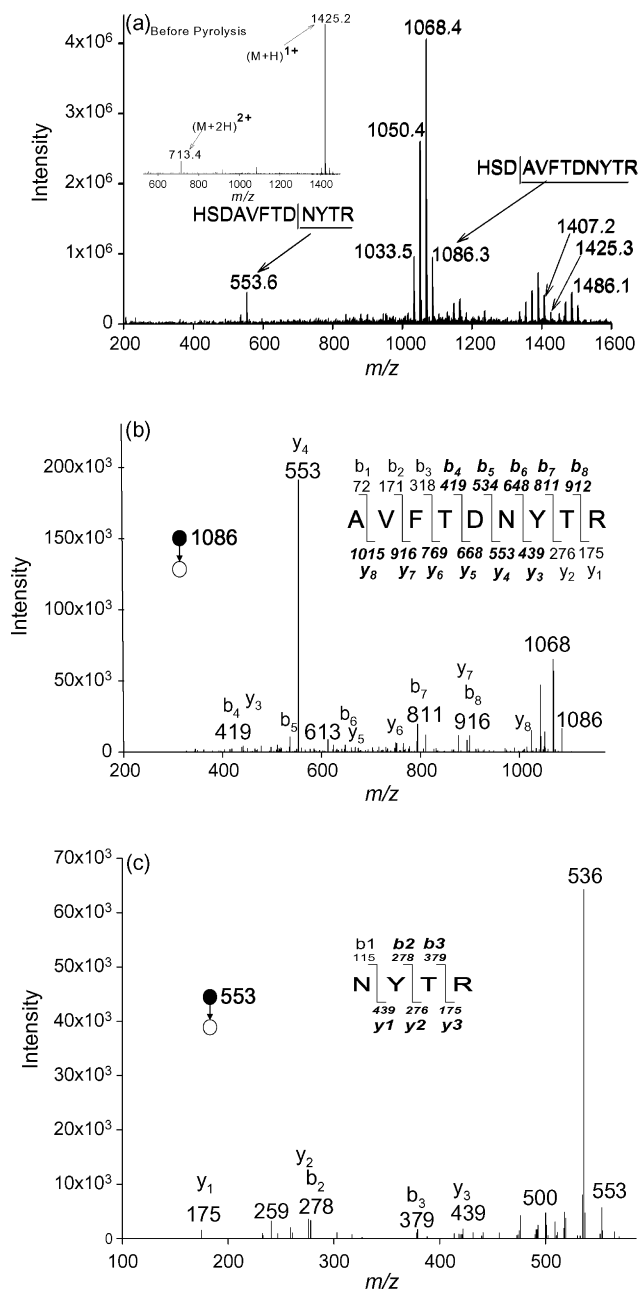


Fig. 3. (a) On-probe pyrolysis DESI-mass spectrum of the VIP peptide showing site-specific cleavages at the two aspartic acids amino acids (inset: before pyrolysis DESI-mass spectrum). On-probe pyrolysis DESI-tandem mass spectrum of pyrolytic product at (b) m/z 1086 and (c) m/z 553.

pyrolysis and the DESI-tandem mass spectrum for the ion at m/z 1201. This ion corresponds to the protein C-terminus peptide due to D-cleavage pyrolysis as confirmed by the DESI-tandem mass spectral data in Fig. 4b. In previous work from our laboratory, it was successfully shown that this sequence information can be used to identify the protein *via* a proteomic-based approach and database search (e.g., MASCOT, Matrix Science Ltd., London, UK) [30].

Fig. 5 illustrates the on-probe pyrolysis DESI-MS analysis of the protein RNase A with the detection of several prominent pyrolysis products observed at m/z 's 437.3, 789.5, 916.4,

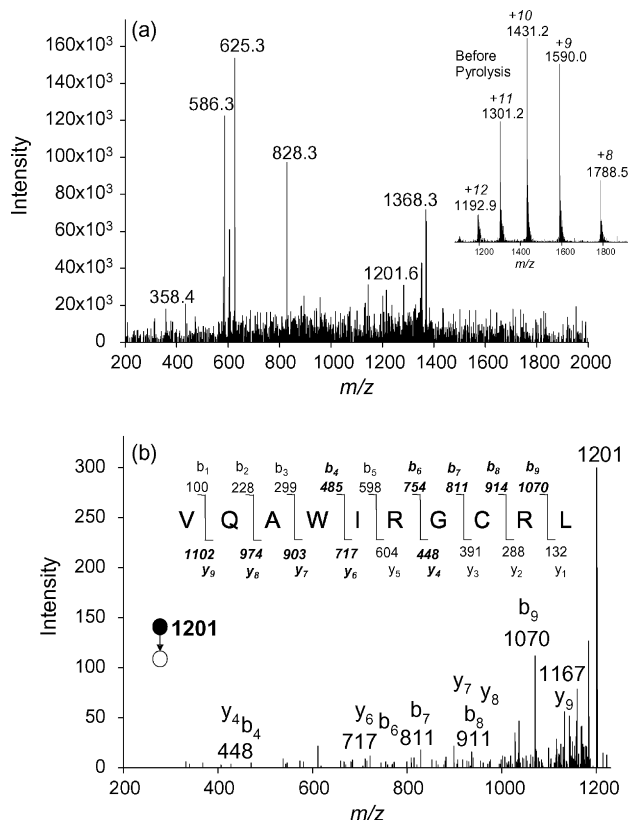


Fig. 4. (a) On-probe pyrolysis DESI-mass spectrum of lysozyme (*inset*: before pyrolysis DESI-mass spectrum), and (b) on-probe pyrolysis DESI-tandem mass spectrum of the ion at m/z 1201.

1047.5 and 1212.4; however, none of the main signals observed match expected products resulting from D-cleavage pyrolysis. In previous investigations and in this study, the D-cleavage pyrolysis peptide product was derived from the C-terminus of the protein sequence, and not from cleavages of internal D groups. While this observation remains to be tested with more protein samples, it might explain the lack of D-cleavage pyrolysis peptides in Fig. 5, since in this case the protein RNase A would generate a small D-cleavage pyrolysis peptide from the C-terminus of the protein at m/z 276 (not detected).

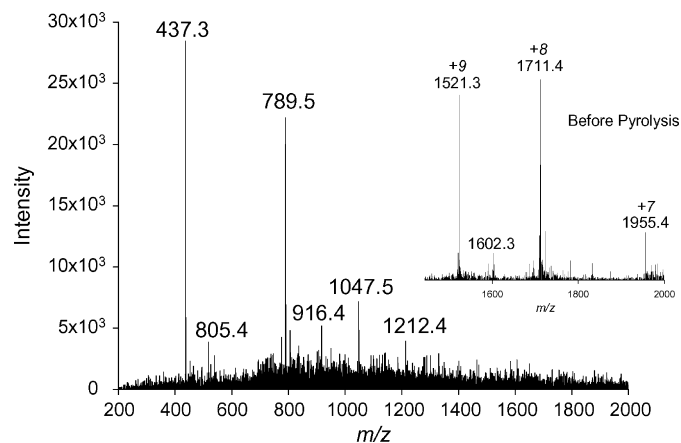


Fig. 5. On-probe pyrolysis DESI-mass spectrum of the protein RNase A (*inset*: before pyrolysis DESI-mass spectrum).

Investigations are currently underway in our laboratory to elucidate the pyrolytic fragmentation pathways for the formation of the unassigned signals in the pyrolysis DESI-mass spectra of these and other proteins using this instrumentation.

3.2. On-probe DESI-MS analysis of poly(ethylene glycol)

Poly(ethylene glycol) with an average molecular weight of 2000 g/mol (PEG 2000) was used to test the ability of the on-probe pyrolyzer DESI-MS instrument to study thermal degradation processes in synthetic polymers. Fig. 6 shows the DESI-mass spectra of the PEG 2000 before and after on-probe pyrolysis at 250 °C for 30 min. The DESI-mass spectrum of untreated PEG 2000 (Fig. 6a) shows a distribution of singly charged ions near m/z 2000 as their $(M+Na)^+$ ions (monomer unit $\Delta m = 44$ u) denoted in the spectrum as the P^+ -series. A doubly charged P^{2+} -series is also observed near m/z 1000 (monomer unit $\Delta m = 22$ u) and is composed of both $(M+2Na)^{2+}$ and $(M+Na+K)^{2+}$ ions [27]. On the other hand, the on-probe pyrolyzed DESI-mass spectrum of PEG 2000 (Fig. 6b and inset) is strikingly different, with the P^+ series shifted to an average molecular weight near m/z 1000, while the P^{2+} and P^{3+}

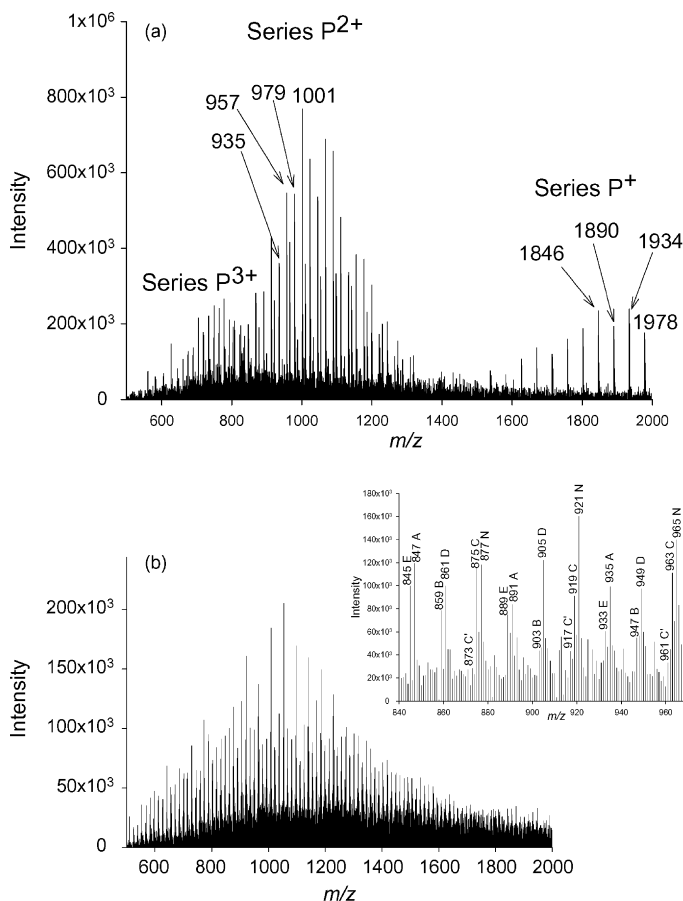


Fig. 6. (a) DESI-mass spectrum of PEG 2000 before pyrolysis, and (b) on-probe pyrolysis (250°C, 30 min) DESI-mass spectrum of PEG 2000. *Inset*: zoomed mass spectrum in the range 840–970 Da. See text for discussion on peak labels.

were not detected. Careful inspection of this mass spectrum (Fig. 6b inset) reveals the presence of several series of poly(ethylene glycol) with different end groups, and these are labeled using nomenclature coined by Voorhees et al. [37]. The spectrum in Fig. 6b is dominated by the unmodified hydroxyl-poly(ethylene glycol) series (labeled N in the spectrum), methyl ether series (A), aldehyde series (C) and the ethyl ether series (D). Less dominant, but present, are the vinyl ether series (B), the methyl ether/aldehyde series (E) and the methyl-vinyl ether series (C'). These results are in direct agreement with previous MALDI-MS studies [9] of the pyrolyzate residues of poly(ethylene glycol), proving that the on-probe pyrolysis DESI-MS technique described in this report yields comparable results. Moreover, the on-probe pyrolysis DESI-MS approach does not require matrix compounds, decreasing sample preparation time and avoiding matrix-sample adducts that can add to the chemical noise in the mass spectrum. Also, in this work, no cationizing agent was added to either the polymer sample or the DESI solvent, and we believe the source of the Na^+ ions to be the glass slide and/or from trace amounts contained in the DESI solvent. It remains to be seen if any sensitivity enhancement is achieved by the addition of a cationizing agent for the analysis of polymers with DESI-MS, either directly to the polymer sample or to the DESI-solvent (reactive-DESI) [38].

4. Conclusion

An on-probe pyrolyzer interfaced with desorption electrospray ionization (DESI) mass spectrometry was successfully demonstrated as a useful tool to detect and analyze non-volatile pyrolysis products of synthetic and biological samples. Our results are in agreement with analyses of non-volatile pyrolysis products performed either by ESI-MS or MALDI-MS, which were pyrolyzed off-line and required sample extraction and solubilization. For biological samples and using the on-probe pyrolyzer DESI-MS system, we have demonstrated that pyrolysis residues of peptides and the protein lysozyme retain sequence information useful for proteomic-based protein identification. Moreover, our results demonstrate that atmospheric pressure pyrolysis can induce a variety of products that include site-specific cleavages at aspartic acid, dehydration reactions in peptides and proteins, and other products of which structures are currently being investigated in our laboratory. For the analysis of poly(ethylene glycol), the on-probe pyrolysis DESI-MS system yielded data and information equivalent to previous MALDI-MS analysis, where the use of a matrix compound and cationizing agent were required. Quantitative to semi-quantitative analysis with DESI-MS is feasible, although quantitation of pyrolysis products was not addressed in this work. Large relative standard deviations ($\sim 30\%$) have been associated with these measurements [16,21] and it was attributed to the solid phase format of the sample in DESI-MS. Overall, results from this work have demonstrated clear advantages of combining an on-probe pyrolyzer with a DESI source that include: minimum sample preparation, no sample extraction or transfer after pyrolysis, atmospheric pressure

pyrolysis, rapid and atmospheric pressure detection by DESI-MS, the ability for sample archival (samples on slides), and tandem-MS (if using a multistage-MS system).

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